

Amendments to the Specification:

Please replace the paragraph beginning at page 19, line 21, with the following rewritten paragraph:

PCR, cloning procedures, and sequencing

Chromosomal DNA from *S.pyogenes* strains was extracted as described in Pitcher *et al*, Lett. App. Microbiol 1989 8 p151-156 modified in the initial incubation step by addition of 1000 U/ml of mutanolysin (Sigma) and 100 mg/ml lysozyme (Sigma). Oligonucleotide primers were designed by using sequence information from the Streptococcal Genome Project database, together with published sequences from other *Streptococcus* spp. Primers 5'-TAG-TAG-CGA-ATT-CGT-CGA-CTG-GCG-CTA-3' (SEQ ID NO 3) and 5'-AGC-ACA-ACT-CGA-GAA-TCG-CTG-TGC-TTT-A-3' (SEQ ID NO 4) enclose almost the whole of *mtsA* (excluding the signal peptide and the NH₂-terminal cysteine residue) and were designed with an *EcoRI* and *XhoI* restriction site, respectively. Primers 5'-GAT-TAC-AAC-TAA-CAA-TCT-TTG-TGT-GAC-C-3' (SEQ ID NO 10), 5'-TTG-ACA-AGG-TAT-CAA-CAG-TAA-ATA-CCT-C-3' (SEQ ID NO 11), 5'-ATG-TCA/T-CTC/T-ATG-GGA/G/T-GAT-GCC-ATC-3' (SEQ ID NO 12), and 5'-TTA/G-GCA-TAT/G-AG/AA-TAA/G-GCC/T-GTC-GC-3' (SEQ ID NO 13) were designed from internal segments of the genes *mtsB* and *mtsC*. PCR experiments were performed using *Taq* polymerase (Gibco-BRL, Gaithersburg, MD), except for cloning purposes, when *TaqPlus* Precision™ (Stratagene, La Jolla, CA) was used. The PCR product corresponding to *mtsA* was gel-purified prior to cloning, using Sephaglas™ Bandprep Kit (Pharmacia Biotech). The PCR profile consisted of 30 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min, followed by a final extension at 72°C for 7 min. Plasmid purification, restriction

enzyme digestions, ligation, electroporation and screening of transformants were all performed according to standard procedures, or when applicable, according to instructions in the GST fusion protein kit (Pharmacia). Sequencing of the cloned insert was performed on an ABI-470 Prism with dyed dideoxy terminators, at Innovagen AB, Lund, Sweden.

Please replace the paragraph beginning at page 26, line 23, with the following rewritten paragraph:

Example 3 - Surface localization of the MtsA lipoprotein

We produced the synthetic peptide QDPHEYEPLPEDV (SEQ ID NO 9), spanning a highly conserved region of MtsA, predicted to have good antigenicity. A rabbit was immunized with the peptide, and following three boosters the serum showed good reactivity with the peptide in ELISA, as compared with preimmune serum (data not shown). Then, a proteolytic digestion of *S. pyogenes* bacteria was performed, to investigate whether a protein fragment from the lipoprotein could be identified using the peptide antiserum. The protein fragments released by proteolytic digestion were subjected to SDS-PAGE and then transferred to PVDF-membrane by Western blotting.

Please replace the paragraph beginning at page 27, line 2, with the following rewritten paragraph:

Immunodetection with the peptide antiserum identified a protein fragment with an apparent molecular mass of 36 kDa, solubilized at high concentrations of papain (data not shown). The protein seemed fairly resistant to proteolytic

digestion, since it remained at the same position even at high papain concentrations when most of the proteins had shifted to the low molecular weight range, supposedly degraded by the excess of protease. Protein from the 36 kDa band was subjected to NH₂-terminal amino acid sequencing. The result (KSDKLKVVAT (SEQ ID NO 14), aa 1-10 of the 36kDa papain fragment) showed a 90% identity to a region very close to the NH₂-terminus in the predicted MtsA protein (amino acids 30-39) of the database (ESDKLKVVAT (SEQ ID NO 15)), and a 100% identity to the predicted MtsA protein (amino acids 30-39) from the sequence of the strain studied (see below). The predicted molecular mass of the mature MtsA polypeptide was 32 kDa. Thus, papain seems to cleave MtsA very close to the protein's predicted NH₂-terminal lipid anchor, liberating almost the whole polypeptide from the bacterial cell surface. A similar papain digestion and western blot was also performed with the strain SF370 sequenced in the Streptococcal Genome Project, with the same result.

Please replace the paragraph beginning at page 31, line 12, with the following rewritten paragraph:

Example 7 - Generation of antiserum

From the genbank MtsA sequence, a number of peptides have been selected based on predicted antigenicity. These are as follows:

Name	Sequence (including terminal C)	length
Spy-LP-TDS21 (259-280)	C*TDSIAKKGKP GDSYYAMMKW N-COOH (<u>SEQ ID NO 8</u>)	22
Spy-LP-ESS16 (234-250)	C*ESSVDRRPME TVSKDS-COOH (<u>SEQ ID NO 7</u>)	17
Spy-LP-KQL17 (136-153)	C*KQLIAKDPKN KETYEKN-COOH (<u>SEQ ID NO 5</u>)	18

Spy-LP-EIN19 (204-223) C*EINTEEEGTP DQISSLIEK-COOH (SEQ ID NO 6) 20

A further peptide, QDP13, has also been highlighted as a potential vaccine candidate, based on its ability to raise antisera in rabbits which reacts with the MtsA protein (data not shown).

QDP13 QDPHEYEPLPEDV (SEQ ID NO 9) 13

The first four peptides have been conjugated to KLH and used to immunize two sheep each as follows: the peptides were derived from peptide synthesis by Genosys Biotechnologies Inc. They are supplied as conjugated lyophilized powder in 0.5mg aliquots in capped vials. The peptides were conjugated to keyhole limpet hemocyanin. C* is a cysteine insert for attachment to a hetero bifunctional linker.